A REVIEW

Selected Advances in Hematology

Ineffective Erythropoiesis; Pathogenesis of Megaloblastic Anemia; Hemostasis and Coagulation

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DURING THE past several years there have been major advances in three areas in the broad field covered by hematology. We have greatly improved our understanding of the concept of ineffective erythropoiesis, the modes of action of vitamin B_{12} and folic acid, and the mechanism of hemostasis and coagulation. These advances require a sharpening of our diagnostic criteria so that improvements in therapy can be fully realized.

Ineffective Erythropoiesis

It is an old clinical observation that patients with full-blown Addisonian pernicious anemia in relapse present something of a paradox with regard to their clinical and laboratory findings. They are usually mildly jaundiced with serum bilirubin levels in the range of 2 to 5 mg per 100 ml and with bone marrow packed full of erythroid precursor cells. The jaundice and erythroid hyperplasia suggests hemolysis as the primary cause of the anemia; however, in contrast to marrow erythroid hyperplasia, the reticulocyte count is decidedly decreased. The quantitative fecal urobilinogen (a measure of the excretion of the degradation products of the heme moiety of the hemoglobin and therefore an index of red blood cell destruction) is three to four times normal. The pronounced increase in fecal urobilinogen suggests that either large numbers of cells are being broken down or that there is an abortive attempt to make hemoglobin in the bone marrow. This paradox of the reticulocytopenia in the presence of erythroid hyperplasia and increased hemoglobin breakdown is very puzzling.[19,32] Similar disturbing observations have been made over the past twenty years in patients with thalassemia and di Guglielmo's disease and in some patients with agnogenic myeloid metaplasia.

The problem became defined when the use of radioactive chromium (Cr^{51}) was brought into clinical medicine as a means of determining red cell life span. This technique involves the *in vitro* labeling of a mixed population of red blood cells which is then injected into the peripheral circulation. In patients with pernicious anemia, a survival of red blood cells approximately one-half of normal was insufficient to explain the four-fold increase in red blood cell destruction indicated by the quantitative determinations of fecal urobilinogen excretion.

The underlying kinetics of the situation were determined by studies with the isotopes, iron 59 (Fe^{59}) and glycine labeled either with carbon 14 (C^{14}) or nitrogen 15. The plasma iron is in equilibrium with the gastrointestinal mucosa, through which iron is absorbed, the bone marrow, to which iron is delivered for hemoglobin manufacture, and iron stores, mainly in the liver and spleen.

Approximately 70 per cent of the iron that passes through the plasma each day is involved in the breakdown and production of hemoglobin. By the use of suitable mathematical models[43] the disappearance of Fe^{59} from the plasma can be used to estimate hemoglobin production. The reappearance of iron in circulating red blood cells is a measure of the effectiveness of the delivery of red cells to the circulation. In normal subjects approximately 75 to 100 per cent of the injected Fe^{59} is incorporated into red blood cells within seven to ten days.

When ferrokinetic studies were done on patients with pernicious anemia in relapse,[19] thalassemia,[52] di Guglielmo's disease[2] some patients with myeloid metaplasia[40] and another group of patients which will be discussed later, the findings were again contradictory. The plasma iron clearance was extremely rapid and indicated that hemoglobin production was four to five times normal, (if the assumptions apply equally to patients and normal controls). However, the incorporation of Fe^{59} into circulating red blood cells seven to ten days after the injection was very low and of the order of 30 per cent. These findings indicated that the intensely erythroblastic marrow in patients with thalassemia

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and pernicious anemia took up iron more rapidly than normal and made hemoglobin more rapidly than normal.

Most of this hemoglobin was not delivered to the peripheral circulation. The use of labeled glycine explained this discrepancy.[33] Glycine is incorporated into both the heme and the globin of hemoglobin. It is given as a single pulse which then labels a single population of red cells.

Isotopically labeled bile pigments derived from heme degradation appear in the stool at two distinct periods. Labeled pigments, accounting for 10 per cent of the administered material, first appear as an "early urobilin peak" approximately four to ten days after injection. Subsequently there is little fecal excretion of labeled pigments until the red blood cells die approximately 120 days later, at which time there is a second peak.

When these techniques were used to study patients with pernicious anemia, there was a huge early urobilin peak accounting for 40 per cent of the administered label, some four to ten days[32] after the isotype was administered. This early excretion of pigments indicated that hemolysis or premature destruction of erythroblasts had taken place before the release of red cells into the circulation.

When we combine the data from clinical, cytologic, and isotopic measurements, the following picture of ineffective erythropoiesis emerges: Because of the anemia, the bone marrow is under a very strong stimulus to make hemoglobin and red cells, and accordingly responds with an intense erythroid hyperplasia and the uptake of large amounts of iron. Sometime during the course of hemoglobin manufacture and erythroid maturation in the marrow the majority of the maturing erythroid cells are irreversibly damaged. This damage might be due to an hereditary defect in the control of hemoglobin synthesis as postulated in thalassemia or might be related to an acquired defect of nucleic acid metabolism due to deficiency of folic acid or vitamin B_{12}. The abnormality results in the premature destruction or hemolysis of erythroblastic cells either within the bone marrow cavity or immediately upon release. This destruction of cells in the marrow cavity, with the concomitant degradation of the hemoglobin and prehemoglobin compounds contained by the cells, leads to the mild bilirubinemia, to increased excretion of fecal urobilinogen and to the large C^{14}-labeled early urobilin peak. There are apparently some erythroid cells in the bone marrow which do not share in the basic defect to the same degree; these cells mature and enter the peripheral blood where they account for the few reticulocytes seen and have a modestly decreased survival. The rapid re-entry of iron from red blood cell breakdown in the marrow plus the accelerated iron absorption from the gut probably accounts for the finding of high serum iron levels and two-thirds saturation of the iron-binding capacity.

There are some investigators who feel that the early appearance of labeled bile pigments in stool is not a result of an abortive attempt to make hemoglobin in the marrow. They suggest that there is a pathway involving a short-cut in bilirubin metabolism which is not dependent on erythropoiesis.[29] This concept has been challenged recently by studies which show that in human subjects erythropoiesis is necessary for bilirubin pigment manufacture.[4]

As techniques for the study of the kinetics of undiagnosed anemias have become more generally available, a heterogeneous group of patients has been observed, all of whom seemed to share the mechanism of ineffective erythropoiesis as the cause of their anemia.[16,23,47] Careful study has shown that these patients do not have pernicious anemia, folic acid deficiency, thalassemia, myeloid metaphasia or di Guglielmo's disease. The patients in this group are ill to a varying degree, but their symptoms are related to the degree of anemia and the extent of secondary hemochromatosis. The anemia is variable from patient to patient and depends on the degree of effective vs. ineffective erythropoiesis. Some of these patients develop the full-fledged picture of secondary hemochromatosis with high serum iron levels and 70 per cent saturation of serum iron binding capacity, hepatic cirrhosis, cardiac abnormalities and pluriglandular disturbances.

The increased iron stores cannot be explained by frequent transfusion or excessive iron feeding, and the iron must have entered the body via increased intestinal absorption. The control of iron absorption seems to depend on three interacting factors[36]: the degree of anemia, the rate of erythropoiesis, and the amount of iron stores. In patients with anemia secondary to ineffective erythropoiesis the iron absorption potentiating effects of the anemia and the greatly increased erythropoiesis overwhelm the inhibitory effects of already increased iron stores, and hemochromatosis develops over a period of years.

Some of those in this group have a fairly uniform clinical picture which has been called *hereditary hypochromic anemia*, and *sideroachrestic anemia*.[16] It seems to be a familial disorder inherited as a sex-linked recessive and presenting as anemia in males in middle life.[48] The peripheral blood shows considerable hypochromia, target cells, and basophilic stippling of red blood cells. There are large amounts of stainable iron in the bone marrow, liver and spleen. There have been attempts to further characterize these patients. Some excrete

large amounts of kynurenic acid in the urine after being given a tryptophane load.[47] Most of the patients have "ringed sideroblasts" in their bone marrow when the Prussian blue stain for iron is used.[10] These cells are normoblasts which contain heavy punctate deposits of iron ringing the cell nucleus, and Bessis has shown that the iron is encrusting the mitochondria.[7]

Some of these patients respond partially with reticulocytosis and increased hemoglobin levels when pharmacologic amounts of pyridoxine[47] or Valentine's liver extract[28] are administered. Splenectomy seems to be of no help. The management revolves about the control of the anemia and the hemochromatosis. Little can be done about the anemia as noted above. The hemochromatosis can be treated, however. Even patients with hemoglobin levels of 10 gm per 100 ml can[16] be dealt with by cautious and repeated phlebotomy, and this group can also be treated with a new iron chelating agent, desferrioxamine B.[37]

We have recently seen two female patients who have hypochromic red blood cells, "megaloblastoid" changes in the marrow, and classical ineffective erythropoiesis, defined kinetically. In both, splenectomy for enlarged spleens has been followed by some improvement in hemoglobin levels but there has been a persistent thrombocytosis of 500,000 to 900,000 per cu mm. Whether these female patients form another group is not yet apparent; however, one patient had a daughter who died at age 21 with hemochromatosis and insulin resistant diabetes.

Pathogenesis of Megaloblastic Anemia

Fundamental studies regarding the mechanism of action of the folic acid and B_{12} vitamins have resulted in a much greater understanding of the pathogenesis of the megaloblastic anemias as well as of their management. Several reviews have recently appeared summarizing the mechanisms of actions of both vitamin B_{12} and folic acid as well as the areas of their interaction.[5,26,38]

Vitamin B_{12}

Vitamin B_{12} occurs in all meats and meat products. In the gut, vitamin B_{12} is bound by a variety of proteins but most avidly by gastric intrinsic factor (IF). The site of the formation of the B_{12}-IF complex is unknown but it seems to take place throughout the small intestine. In its passage through the small bowel, uncomplexed vitamin B_{12} can also be utilized by bacteria and parasites. At the ileum,[8,17] possibly under the control of intestinal[49] or pancreatic secretion,[55] the vitamin B_{12}-IF complex is bound to the wall of the ileum, and partial absorption of the complex begins. Dissociation of the complex then occurs and vitamin B_{12} completes its passage through the ileal wall. It is thought by some investigators that a specific intestinal releasing factor is required to open the IF-B_{12} complex.[25] The absorbed vitamin B_{12} is carried by vitamin B_{12}-binding serum proteins throughout the body. Relatively large amounts go to the liver and marrow. The normal liver can store 1000 to 1500 micrograms of vitamin B_{12}, so that at a rate of utilization of 1 to 6 micrograms vitamin B_{12} per day the liver contains a 1.5 to 4 year depot.[21]

Vitamin B_{12} acts as a co-enzyme in many living forms, but probably its major function in mammals is its role in deoxyribonucleotide (DNA) synthesis.[5,57] The specific mode of action of vitamin B_{12} in DNA synthesis is not clear, but it is well known that DNA is required for cell division. The only known specific site of action of B_{12} co-enzyme in mammalian tissue involves a sequence in propionic acid metabolism in which methyl-malonyl CoA is converted into succinyl CoA.[5]

From the foregoing it can be seen that vitamin B_{12} deficiency states can result from a variety of causes. The most common and well-known is classical Addisonian pernicious anemia in which the specific deficiency of gastric intrinsic factor leads to an inability to absorb vitamin B_{12} from the diet. Most often pernicious anemia appears in the sixth decade when it is associated with gastric atrophy and histamine-fast achlorhydria even when tested with the augmented histamine test.[58]

There is some evidence that pernicious anemia may be a genetically conditioned disorder. Pernicious anemia has now occurred in several children who are presumed to be "homozygous." They have no gastric intrinsic factor but, on biopsy have normal gastric histology and also normal amounts of hydrochloric acid.[56]

Other causes of vitamin B_{12} deficiency can be anticipated from consideration of its absorption and metabolism. It may occur in a group of food faddists called "vegans" who eat no animal protein and whose diets are therefore deficient in vitamin B_{12}. Superabundance of small intestinal bacterial growth can by competing successfully for the vitamin B_{12} in the gut, result in vitamin B_{12} deficiency. This form of bacterial overgrowth occurs most commonly when there are multiple small bowel diverticula or when blind loops of small bowel have been created by surgical operation or trauma.[22,51] Parasitism of the gut can also result in vitamin B_{12} deficiency, the classic example being infestation of the gut with the fish tapeworm (D. latum) as seen in Finland.

Since a relatively intact ileum is needed for vitamin B_{12} absorption, a variety of malabsorptive diseases can result in vitamin B_{12} deficiency.[22,38] However, in nontropical sprue, which rarely involves

the ileum, there is malabsorption of vitamin B_{12}. The explanation may reside in the role played by intestinal secretions which may have a releasing factor action.[49] Several patients with pancreatic insufficiency and steatorrhea have been reported to exhibit malabsorption of vitamin B_{12}. An alkaline pH is necessary for proper absorption of vitamin B_{12} and the sodium bicarbonate content of the pancreatic exocrine secretion contributes greatly to the maintenance of the alkaline pH. Addition of sodium bicarbonate partially corrected the malabsorption of vitamin B_{12} in some patients with pancreatic insufficiency, but there was a further increase in absorption when pancreatin was added as well, indicating a possible role of pancreatic enzymes in vitamin B_{12} absorption.

Deficiency of vitamin B_{12} from any cause results in a variable clinical picture which can be manifested by disorders in three organ systems.

1. There is glossitis, indigestion and diarrhea.

2. Hematologically there is a progressive and severe anemia which has the dynamics of ineffective erythropoiesis. In addition to ineffective erythropoiesis the primitive megaloblasts in the marrow have a prolonged maturation time.[41] The peripheral blood shows distorted red blood cells, macro-ovalocytosis and nucleated red cells. There may be a granulocytopenia with hypersegmented neutrophils, and the platelets may be decidedly decreased. The marrow shows classical megaloblastic erythroid hyperplasia. If the patient has a coincident iron deficiency[38] the megaloblastic features may be masked. Granulocyte precursors and megakaryocytes are decreased in number and there are usually giant metamyelocytes. Iron stores in marrow are frequently increased.

3. Vitamin B_{12} deprivation also leads to the neurologic picture of combined system disease which consists of peripheral neuropathy and spinal cord degeneration.

The etiology of pernicious anemia remains obscure. The previously mentioned familial incidence has led to the concept that the disease may be genetically conditioned. Several laboratories have now reported the finding, in 50 per cent of patients with pernicious anemia, of a circulating antibody to intrinsic factor which can block the absorption of vitamin B_{12}.[54] The findings suggest that pernicious anemia may be the result of immune mechanisms directed against the action or production of intrinsic factor. If this is the etiology of pernicious anemia, the patient with juvenile pernicious anemia (who has no gastric atrophy or achlorhydria) should have the antibody in his serum. Of ten such children tested thus far, none have an antibody to intrinsic factor.[53]

Folic Acid

Deficiency of folic acid results in a clinical picture which has many similarities with vitamin B_{12} deficiency. The same megaloblastic anemia characterized by ineffective erythropoiesis is induced. There may also be leukopenia and thrombocytopenia. The red cells in the peripheral smear are macrocytic and there is hypersegmentation of the polymorphonuclear cells. Combined system disease does not occur.

The action of folic acid centers about the transfer of a variety of single carbon groups,[20,26,38] which are required for purine ring closure and in the conversion of deoxyuridylic acid to thymidylic acid. Folic acid is therefore important in nucleic acid metabolism and cell division.

Folic acid and related compounds are contained in animal organs and fresh green vegetables, but can be destroyed and diluted by boiling in large amounts of water.[24] Absorption occurs in the upper small bowel and the body seems to be unable to store a large amount of folic acid so that deficiency states may appear more rapidly than in the case of vitamin B_{12} deficiencies.[24] The normal daily requirement of folic acid is 100 to 200 micrograms per day.[11,24] However, certain stress situations can increase the requirements.

Deficiency of folic acid can be seen on a dietary basis in alcoholic persons and food faddists. Since a relatively intact small intestine is required for the absorption of folic acid, deficiency can occur in a variety of intestinal malabsorptive syndromes, including tropical sprue, regional enteritis, and idiopathic steatorrhea.[20]

Recently a variety of conditions have been identified in which there is an increased requirement for folic acid. In certain chronic hemolytic states and perhaps pregnancy, a normal diet may not contain enough folic acid. The hemolytic anemias in which superimposed folic acid deficiency has been reported include sickle cell anemia, thalassemia, and acquired hemolytic anemia.[12,31] The clinical picture generally shows the chronic anemia becoming more severe with increasing transfusion requirements and falling reticulocyte counts. Megaloblastic changes in the marrow may be present or absent. Administration of folic acid results in restoration of the hemoglobin and the reticulocyte count to previous levels. One patient with sickle cell anemia required 1000 micrograms of folic acid per day (five to ten times the normal requirement) before the marrow became normoblastic.[31]

Lastly, there are two classes of drugs which can interfere with the utilization of folic acid. The use of the antifols, aminopterin and amethopterin, in the treatment of acute leukemia is based on their ability to impair folic acid metabolism by blocking the conversion of folic acid to metabolically useful

forms. Prolonged use of these drugs can result in folic acid deficiency, although the acute leukemia ordinarily does not permit of prolonged therapy. The anticonvulsants dilantin and mysoline occasionally cause an anemia due to folic acid deficiency. Long term treatment at high dose levels increases the chance of anemia. The blood folic acid levels are decreased even in the presence of a good diet, suggesting that anticonvulsants impair metabolism of folic acid due to certain shared structural similarities.[30]

A major advance in hematology has been in the methods used in determining whether a deficiency of vitamin B_{12} or folic acid is causing the megaloblastic anemia. The implications of one or the other deficiency state are quite different. Thus Addisonian pernicious anemia is associated with an increased incidence of gastric cancer and the management of the patient must include periodic searches for gastric cancer. Megaloblastic anemia due to vitamin B_{12} deficiency may be the first sign of a relapse of regional ileitis.

Folic acid deficiency may occur in idiopathic steatorrhea, chronic alcoholism, chronic hemolytic anemia or drug therapy. The differential diagnosis can be made by reviewing the family history and by questioning regarding drug intake, weight, bowel function, and previous abdominal operations or abdominal trauma. The cytologic examination of peripheral blood and marrow is not particularly helpful in distinguishing the two.

There are now available sensitive microbiological assays for measuring folic acid and vitamin B_{12} levels in plasma or serum. When available these tests constitute the simplest and most direct way of determining which vitamin is deficient. However, the complexity of these methods indicates that they will probably not reach routine clinical use for many years. The distribution and excretion rates of labeled folic acid provide a measure of folic acid stores, as does the measurement of formiminoglutamic acid excretion in the urine after a loading dose of histidine. These methods are more suitable for special laboratories and are beyond the scope of this article.[38]

There are, however, two methods generally available. One involves the use of the cobalt radioisotopes of vitamin B_{12} in the Schilling test, which is a method for estimating vitamin B_{12} absorption. A tracer dose of labeled vitamin B_{12} is given orally and this is followed by a large parenteral dose of unlabeled vitamin B_{12}. This "tide" of unlabeled vitamin B_{12} spills over into the urine, carrying with it a proportion of the labeled vitamin B_{12} which has been absorbed from the gastrointestinal tract. The radioactivity in the urine is then determined. The reticulocyte response to the large flushing dose of vitamin B_{12} can be monitored, and it provides a rough (and occasionally misleading) index of response.[59] If the Schilling test result is initially abnormal in the presence of good renal function, it should be repeated with added oral intrinsic factor. If added intrinsic factor brings vitamin B_{12} absorption to normal, the patient has Addisonian pernicious anemia due to deficiency of intrinsic factor. If the test is still abnormal, the patient has an absorptive defect. Administration of broad spectrum antibiotics before a third Schilling test is useful if one suspects that the patient has vitamin B_{12} deficiency secondary to jejunal diverticula or intestinal blind loops.[22]

THE disadvantage of the Schilling test is that it requires doses of vitamin B_{12} which can cause a reticulocyte response even in patients with folic acid deficiency. Furthermore good renal function and accurate urine collections are necessary.[59]

The simplest tests are based on the parenteral administration of small physiological doses of each vitamin, accompanied by serial measurements of the reticulocyte response. At the doses chosen, a reticulocyte response indicates a deficiency of that vitamin. In general, one first selects the vitamin which one thinks is not at fault. Therefore if one judges on the basis of clinical data that a patient has folic acid deficiency, one would begin the test by measuring daily reticulocyte counts, and starting treatment with one microgram of vitamin B_{12} parenterally per day. If a patient has a vitamin B_{12} deficiency state not obscured by iron deficiency or concurrent infection, there will be a pronounced rise in reticulocytes within five to seven days. If after ten days there is no response to vitamin B_{12}, trial administrations of 100 to 200 micrograms of folic acid parenterally per day should be started and maintained for ten days. If the deficiency is due to this vitamin, in five to seven days there will be an increase in reticulocytes.

It should be emphasized that larger doses of either vitamin will cause a misleading reticulocyte response. Thus patients with folic acid deficiency will respond to 500 micrograms of B_{12} per day while patients with vitamin B_{12} deficiency states will respond to 400 micrograms of folic acid per day.[24,59] The therapeutic response tests take time and should not be used in acutely ill patients who have neurologic disease or severe thrombocytopenia or leukopenia.[24]

A test which may become clinically available utilizes the specific action of vitamin B_{12} as the coenzyme in the conversion of methyl-malonyl CoA to succinyl CoA. A deficiency of vitamin B_{12} should result in a piling up of methyl malonate in urine, and

such is indeed the case.[3] Patients with folic acid deficiency have normal excretion.

The dose and route of administration of vitamin B_{12} and folic acid are dictated by the causes of the deficiency state. The therapy of megaloblastic anemia has been recently reviewed.[24] There is no evidence that vitamin B_{12} or folic acid has any therapeutic use other than in the treatment of the specific deficiency states.

Advances in Hemostasis and Coagulation

Discussion here of advances in hemostasis and coagulation will be limited to two aspects of this subject: The recent availability of concentrated human AHG (anti-hemophilic globulin, Factor VIII) preparations for the treatment of classical hemophilia, and the current status of our knowledge of platelet life-span and function in hemostasis.

Deficiency of Factor VIII or anti-hemophilic globulin accounts for approximately 80 per cent of cases of hemophilia. Our improved ability to diagnose and manage hemophilia derives from: (1) a clearer idea of the function and biological half-life of Factor VIII in first stage coagulation,[44] (2) the emergence of specific assays for Factor VIII,[46] and (3) the development by several pharmaceutical companies* of concentrated preparations of human AHG.[35,45] These preparations not only contain increased amounts of AHG per volume but have a sharply reduced content of extraneous protein so that we can now give infusions of AHG which would previously have led to overloading of the circulation and to noncardiac circulatory congestion. With these preparations it is now possible to raise the AHG levels from 1 per cent of normal in moderately severe hemophilia to 30, 50 and even 100 per cent of normal.[45] Therefore, severe bleeding which was not controllable in the past with fresh lyophilized plasma can now be managed. It is even possible to consider elective but necessary surgical operation in properly prepared hemophiliac patients.

Several precautions must be taken however, in the use of these preparations:

- They are not indicated in the more routine hemorrhagic complications of hemophilia because of their great cost. Use should be limited to serious intracranial, intraabdominal, retroperitoneal, and retropharyngeal bleeding.

- The preparations should only be used in conjunction with a laboratory which can perform one of the specific AHG assays The calculations of dose required are based on AHG assays of the patient's plasma (along with actual measurements of the patient's plasma volume) and the preparation to be used. Serial measurements of the AHG levels achieved are then made. Use of the AHG concentrate without laboratory control will result either in inadequate therapy, which is dangerous, or in overuse, which is wasteful.

- Use of the preparation should be preceded by a specific assay for anti-AHG anticoagulants in the patient's blood. Use of AHG concentrates when the patient has a specific AHG anticoagulant is not only wasteful but dangerous, in that it may stimulate the production of larger amounts of anticoagulant.

- Use of the preparations in severe hemorrhage is a technical *tour de force.* Infusions are required every six to eight hours, with monitoring of the patient's general condition and plasma AHG levels. The duration of infusions is not definitely established but, in general, AHG levels of 30 per cent should be maintained for 48 to 72 hours after subsidence of symptoms. In the case of general abdominal surgical operations, there have been reports of severe hemorrhage occurring a week after operation, possibly related to wound healing and capillary regrowth. Therefore, it seems prudent to maintain infusions for seven to ten and even fourteen days following operation.[35]

Platelet Function

Studies on platelet function have contributed much to our understanding of the steps involved in hemostasis. Microcinematographic investigations of platelets in small vessels[6] have revealed the following sequence: When a small wound is made in a capillary vessel, there is an immediate piling up of platelets as in a snowdrift. The platelets adhere to and aggregate at the subendothelial portion of the break, and to one another. However, they do not completely impede the passage of blood out of the vessel.

The trigger mechanism for platelet aggregation *in vivo* is unknown, but several recent studies have shown that adenosine diphosphate and epinephrine can cause platelet aggregation *in vivo* and *in vitro.*[9,18,42] After 30 seconds there is a thrombin-mediated change in the physical characteristics of the platelet aggregate which is called viscous metamorphosis (the source of the thrombin for this reaction remains obscure). The platelets swell and when this occurs the platelet aggregate becomes a platelet plug that is no longer permeable to red cells, whereupon bleeding through the opening in the vessel wall stops.[50] When the platelets swell they also expel platelet factor 3, a substance necessary for thromboplastin generation.[44] Subsequently, red cells and more platelets become entrapped behind this platelet plug and coagulaton takes place. The platelets appear to be spaced regularly throughout the clot at the intersection of fibrin strands and they produce clot retraction by sending out long dendritic processes which pull on these intersecting strands.

*Merck, Sharp & Dohme, and Cutter Laboratories.

Platelets are now known to have a contractile protein similar to muscle actomyosin.[34] The bulky clot is therefore pulled into a small tough patch covering the break in the endothelium.

Studies on platelet life-span are becoming available now that some of the technical problems of platelet labeling are being solved.[1,13,39] Reports thus far indicate that most patients with acute idiopathic thrombcytopenic purpura (ITP) have profound shortening of platelet survival.[39] There is a report that some patients with chronic ITP have slight shortening of platelet survival but a more serious impairment of platelet production. One patient in this group improved after splenectomy.[13]

In contrast, patients with thrombocytopenia due to hypersplenism have generally shown a rather normal platelet survival indicating that the thrombocytopenia is induced by a platelet production defect.[39] Removal of the spleen in some of these patients has resulted in a return in platelet count to normal with the platelet survival remaining normal.[14]

These observations raise again the possibility, discarded over the last few years, that the spleen may exert a humoral depressant effect on megakaryocytes and that removal of the spleen removes this source of humoral suppression. In the presence of enlargement of the spleen, this suppressive effect becomes intensified and can be corrected by the surgical removal of the spleen.[15,27]

Comment

Although recent advances in hematology have greatly improved our understanding of the kinetics and sites of red blood cell destruction and production, we are, for the most part, still ignorant of the underlying causes of the destruction. How deficiency of vitamin B_{12} leads to neurological disease is still not known. We are a long way from being able to protect a hemophiliac patient from hemorrhagic episodes by a simple injection that could be given several times weekly. These questions are under active attack in several laboratories and perhaps the next few years will supply some answers.

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